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Method development and validation for simulataneous estimation of albendazole and praziquantel in bulk and in a synthetic mixture

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Abstract

A simple, rapid, sensitive reversed-phase high-performance liquid chromatography method was developed and validated for simultaneous measurement of albendazole and praziquantel with an internal standard, simvastatin, at single wavelength of 225 nm. Chromatographic separation was performed on an Enable C_{18} column (250 mm × 4.6 mm, 5 μm: Spinco Biotech Pvt Ltd) and a mobile phase consisting of acetonitrile:water (60:40, v/v) with 10% orthophosphoric acid to adjust the pH to 3.2, at a flow rate of 1.0 ml/min. The calibration curve was linear ($r^2 \geq 0.999$) over the concentration range 0.05–8.0 μg/ml. The concentrations of simvastatin was 1.0 μg/ml. The limit of quantification was 0.05 μg/ml for both albendazole and praziquantel. No interference was found by the excipients in the synthetic mixture. The proposed methods were validated as per International Conference on Harmonisation guidelines for linearity, accuracy, precision and robustness for estimation of albendazole and praziquantel in bulk and in a synthetic mixture, and the results were found to be satisfactory.

Keywords: Albendazole; Praziquantel; Simvastatin; HPLC; UV detection; Validation

1. Introduction

Neurocysticercosis is the commonest helminthic disease of the nervous system and is considered a serious public health problem in developing countries of Latin America, Asia, and Africa [1–3]. Although the treatment of neurocysticercosis is restricted to palliative measures, it has advanced over the past 20 years with the use of praziquantel and albendazole, which are effective against the cystic larvae [2,4]. Albendazole is more effective than praziquantel, but cysts persist in some patients even after repeated use of albendazole [2]. For these cases, alternative treatment schedules such as simultaneous use of praziquantel and albendazole have been evaluated [4,5]. The combination of praziquantel with albendazole has also been extensively used in human hydatid disease [6–9]. Albendazole is extensively metabolized to its active metabolite albendazole sulfoxide, which is further metabolized to the inactive albendazole sulfone [10]. Because of this extensive metabolism, plasma concentrations of albendazole are usually low, and pharmacokinetics are studied by measuring the sulfoxide and sulfone concentrations [11–15]. Praziquantel is metabolized to several hydroxylated metabolites [16–18], mainly *trans*-4-hydroxypraziquantel, an active metabolite [19].

In order to evaluate the kinetics of albendazole and praziquantel, selective, sensitive, reproducible analytical methods are required for their quantification in plasma samples as well as for their metabolites. High-performance liquid chromatography (HPLC) [20–29] and capillary electrophoresis [30,31] have been used,

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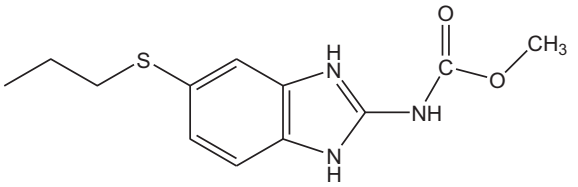

Fig. 1. Chemical structure of albendazole.

and the coupling of mass spectrometry to liquid chromatography (LC–MS and LC–MS–MS) has brought new insight into quantitative bioanalysis. Use of these techniques for the analysis of albendazole metabolites, praziquantel and *trans*-4-hydroxypraziquantel has been described only for isolated drugs. Bonato et al. [32] and Chen et al. [33] reported the use of LC–MS–MS for two methods, with quantification limits for albendazole sulfoxide of 5.0 and 4.0 μg/ml, respectively, and a quantification limit of 0.5 μg/ml for albendazole sulfone. LC–MS–MS was used only for qualitative analysis of praziquantel metabolites [16,34].

The aim of this work was to develop an HPLC method for simultaneous estimation of albendazole and praziquantel in bulk and in the synthetic mixture. The method was validated according to the International Conference on Harmonisation guidelines.

2. Materials and methods

2.1. Chemicals and reagents

Albendazole (Fig. 1) was obtained from Mercury Pharmaceutical Ltd, Vadodara, Gujarat, India, praziquantel (Fig. 2) from Micro Labs Ltd, Goa, India, and simvastatin (internal standard) (Fig. 3) from Dr Reddy's Lab, Hyderabad, India. Acetonitrile, methanol and water of HPLC grade were used. All the other reagents (including 10% *ortho*-phosphoric acid) were of analytical grade.

2.2. Chromatographic conditions

The high-performance liquid chromatograph (Shimadzu, Kyoto, Japan) was composed of an LC-20AT

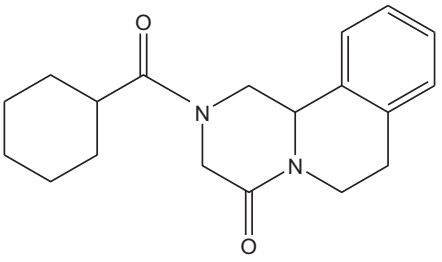

Fig. 2. Chemical structure of praziquantel.

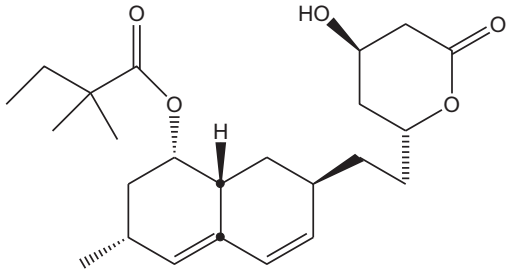

Fig. 3. Chemical structure of simvastatin (IS).

Prominence solvent delivery module, a manual rheodyne injector with a 20-μl fixed loop and a SPD-20A Prominence ultraviolet–visible detector. Separation was performed on an Enable C18 G column (particle size 5 μm; 250 mm × 4.6 mm) preceded by an ODS guard column (10 μm, 10 mm × 5 mm) at ambient temperature. Data were acquired on a Spinchrom Chromatographic Station® CFR Version 2.4.0.195 (Spinchrom Pvt. Ltd, Chennai, India). The mobile phase consisted of acetonitrile:water in a ratio of 60:40, the pH was adjusted to 3.2 with *ortho*-phosphoric acid, and the flow rate was 1.0 ml/min. Mo was vacuum filtered and degassed through 0.2 μm pore polymeric PTFE filters.

2.3. Method

2.3.1. Preparation of standard stock solutions

A sample of 25 mg of each drug is weighed and transferred to a 25-ml volumetric flask; 15 ml of methanol are added, and the solution is sonicated for 15 min. The volume is made up to the mark with methanol to obtain a stock solution of 1000 μg/ml. Simvastatin is also prepared in the diluent to obtain a working standard solution of 1000 μg/ml.

2.3.2. Preparation of working standard solutions

From the standard stock solutions, 2.5 ml are withdrawn and transferred to 25-ml volumetric flasks, and the volume is made up to the mark with diluent to obtain working standard solutions of 100 μg/ml. The working standard solution of simvastatin is diluted to a final solution of 10 μg/ml.

2.4. Validation

The method was validated by evaluating recovery, linearity, precision, accuracy, quantification limit and stability. Coefficients of variation and relative errors <2% were considered acceptable [35,36].

2.4.1. Linearity

Linearity was tested at concentrations of 8, 7, 6, 5, 4, 3, 2, 1, 0.5 and 0.05 μg/ml for albendazole and praziquantel with a fixed concentration of 1 μg/ml diluted from 10 μg/ml of the working standard solution. The calibration curve was constructed and its coefficient of determination (r^2) determined. The calibration plot (peak area ratio of albendazole and praziquantel to internal standard versus albendazole and praziquantel concentration) was generated by replicate analysis ($n = 10$) at all concentrations, and the linear relation was evaluated by the least-squares method in Microsoft Excel®. For minimum error, 10 concentrations were tested, giving a wide range of linearity.

2.4.2. Accuracy

The accuracy of the method was determined by replicate analyses with two standard addition methods at six concentrations, the first at 80%, 100% and 120% and the second at 50%, 100% and 150%. Comparison of the difference between the spiked value (theoretical value) and that found gave the accuracy.

2.4.3. Precision

The precision of the method, measured as within-day repeatability, was determined by replicate analyses of three sets of samples spiked with three concentrations of albendazole and praziquantel (0.05, 1 and 8 μg/ml) and a fixed concentration of internal standard (1 μg/ml). The reproducibility (day-to-day variation) of the method was validated with the same concentration range, but with only a single determination of each concentration on three days. The relative standard deviation was calculated from the ratio of the standard deviation to the mean and expressed as a percentage.

2.4.4. Specificity

Specificity was measured by analysing the standard solutions in the presence of an excipient (talc) to determine any interference in the percentage recovery. Standard solutions containing 10 mg of each drug were spiked with 50% (5 mg), 100% (10 mg) or 150% (15 mg) of talc and analyzed for albendazole and praziquantel recovery by HPLC with a fixed concentration of internal standard (1 μg/ml). The acceptable level of interference was <0.5%.

2.4.5. Robustness

The robustness of the method for determining albendazole and praziquantel was evaluated by varying the flow rate, pH and mobile phase ratio. The percentage recovery and relative standard deviation were recorded for both drugs.

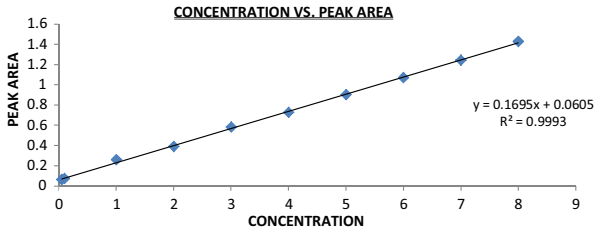

Fig. 4. Calibration curve of praziquantel.

3. Results and discussion

3.1. Calibration curve

The coefficient correlation r, slope and intercept were 0.999, 1.064 and 0.358 for albendazole and 0.999, 0.169 and 0.060 for praziquantel with ultraviolet detection (Fig. 6) with absorbance maxima set at 225 nm. The retention times were 4.533 for albendazole, 6.39 for praziquantel and 7.8 for the internal standard (Fig. 7). Linear regression of data from the calibration curve indicated a linear response over the concentration range of both drugs. The curve can therefore be used for determination of albendazole and praziquantel in synthetic mixtures.

3.2. Validation

3.2.1. Linearity

The coefficient of determination (r^2) for both albendazole and praziquantel was 0.999 (Tables 1 and 2 and Figs. 4 and 5).

3.2.2. Limit of detection and limit of quantification

The limit of detection and the limit of quantification were determined from the calibration curve, according to the formulae 3.3 × SD/slope and 10 × SD/slope, respectively. The results are shown in Table 2.

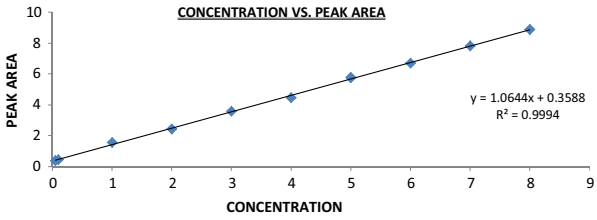

Fig. 5. Calibration curve of albendazole.

Table 1
Linear regression equations generated from validation of albendazole and praziquantel: slope, intercept and coefficient of determination.

Analyte					
Praziquantel			Albendazole		
Conc. (μg/ml)	Peak area (mV.s)		Conc. (μg/ml)	Peak Area (mV.s)	
0.05	0.060	Slope: 0.169	0.05	0.374	Slope: 1.064
0.1	0.071		0.1	0.442	
1	0.257		1	1.550	
2	0.388	Intercept: 0.060	2	2.440	Intercept: 0.358
3	0.580		3	3.601	
4	0.728		4	4.470	
5	0.903		5	5.770	
6	1.069	r^2: 0.999	6	6.712	r^2: 0.999
7	1.244		7	7.814	
8	1.427		8	8.888	

Table 2
Spectral and statistical data for determination of albendazole and praziquantel by proposed RP-HPLC method.

Analyte			
Praziquantel		Albendazole	
Absorption maxima, λ (nm)	225	Absorption maxima, λ (nm)	225
Linearity range (μg/ml)	0.05–8	Linearity range (μg/ml)	0.05–8
Coefficient of determination (r^2)	0.999	Coefficient of determination (r^2)	0.9991
Regression equation (Y[a])	$Y = 0.169x + 0.060$	Regression equation (Y[a])	$Y = 1.064x + 0.358$
Slope (b)	0.169	Slope (b)	1.064
Intercept (a)	0.060	Intercept (a)	0.358
Limit of detection, LOD (μg/ml)	0.0167	Limit of detection, LOD (μg/ml)	0.0167
Limit of quantitation, LOQ (μg/ml)	0.05	Limit of quantitation, LOQ (μg/ml)	0.05

[a] $Y = mx + c$, where x is the concentration (μg/ml).

3.2.3. Precision

The relative standard deviation was found to be <2.0% for both albendazole and praziquantel, indicating satisfactory precision (Table 3). The intermediate precision of the expected results is expressed as a percentage.

3.2.4. Accuracy

The mean recovery of praziquantel and albendazole was found to be in the range 100.81–100.92% and 99.71–100.86%, respectively, within the acceptable limits at 80%, 100% and 120% (Table 4a) and

Table 3a
Intra-day ($n = 3$) precision.

Analyte									
Albendazole					Praziquantel				
Conc. (μg/ml)	Conc. found at 4.5 (μg/ml)	Avg. (μg/ml)	S.D	% RSD	Conc. (μg/ml)	Conc. found at 6.38 (μg/ml)	Avg. (μg/ml)	S.D	% RSD[a]
0.05	0.049				0.05	0.499			
0.05	0.0501	0.0496	0.0005	1.179	0.05	0.051	0.050	0.0007	1.377
0.05	0.0499				0.05	0.049			
4	3.914				4	3.995			
4	3.874	3.928	0.063	1.610	4	4.09	4.031	0.051	1.277
4	3.998				4	4.008			
8	7.975				8	7.944			
8	8.098	7.987	0.105	1.315	8	8.05	7.966	0.075	0.941
8	7.80				8	7.905			

[a] % RSD = SD/mean × 100.

Table 3b
Inter-day ($n = 3$) precision.

Analyte									
Albendazole					Praziquantel				
Conc. (μg/ml)	Conc. found at 4.5 (μg/ml)	Avg. (μg/ml)	S.D	% RSD	Conc. (μg/ml)	Conc. found at 6.38 (μg/ml)	Avg. (μg/ml)	S.D	% RSD[a]
0.05	0.051				0.05	0.052			
0.05	0.052	0.052	0.0009	1.82	0.05	0.050	0.051	0.0009	1.857
0.05	0.051				0.05	0.051			
4	4.104				4	4.115			
4	4.259	4.18	0.078	1.880	4	4.109	4.147	0.061	1.477
4	4.206				4	4.218			
8	8.299				8	8.104			
8	8.098	8.142	0.140	1.722	8	8.211	8.106	0.103	1.277
8	8.029				8	8.004			

[a] % RSD = SD/mean × 100.

Table 3c
Repeatability.

Analyte									
Albendazole					Praziquantel				
Conc. (μg/ml)	Conc. found at 4.5 (μg/ml)	Avg. (μg/ml)	S.D	% RSD	Conc. (μg/ml)	Conc. found at 6.38 (μg/ml)	Avg. (μg/ml)	S.D	% RSD[a]
4	3.994				4	4.115			
4	3.974				4	4.109			
4	3.998	4.039	0.058	1.44	4	4.018	4.055	0.054	1.34
4	4.104				4	3.995			
4	4.059				4	4.09			
4	4.106				4	4.008			

[a] % RSD = SD/mean × 100.

98.33–100.66% and 98.46–100.73% within the acceptable limits at 50%, 100% and 150% (Table 4b).

3.2.5. *Specificity*

Interference was found to be 100.81–100.92% for albendazole and 100.39–100.89% for praziquantel, which is within the acceptable limit. Hence the excipients do not interfere with estimates of drug concentrations (Table 5).

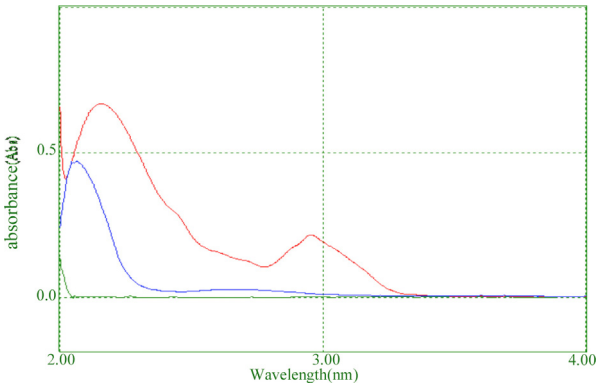

Fig. 6. UV spectra of ALZ & PRQ with blank (methanol) (6 μg/ml).

3.2.6. *Robustness*

The retention times of the analytes did not change significantly when the flow rate, mobile phase ratio and pH were changed. The percentage recovery and relative standard deviation were within the limits for both albendazole and praziquantel (Tables 6 and 7).

3.2.7. *Sample stock solutions for assay*

Ten tablets powdered equivalent were mixed in a ratio of 300 mg albendazole: 25 mg praziquantel. A quantity of this synthetic mixture powder equivalent to 65 mg was taken up in a 100-ml volumetric flask, and diluent was added up to the mark. The solution was sonicated for 5 min. This solution was further diluted to obtain a concentration of 12 μg/ml albendazole and 5 μg/ml

Table 4a
Accuracy.

Analyte	% Added	Constant amount added[a] (μg/ml)	Amount added[b] (μg/ml)	Total amount found[c] (μg/ml)	Amount found[d] (μg/ml)	% Recovery[e]	Average % recovery	% RSD
PRZ (3 μg/ml)	80	3	2.4	5.391	2.391	99.65		
	80	3	2.4	5.403	2.403	100.13	99.95	0.263
	80	3	2.4	5.401	2.401	100.08		
	100	3	3	5.958	2.958	98.63		
	100	3	3	5.974	2.974	99.14	99.38	0.903
	100	3	3	6.011	3.011	100.37		
	120	3	3.6	6.603	3.603	100.09		
	120	3	3.6	6.582	3.582	99.50	100.09	0.499
	120	3	3.6	6.617	3.617	100.49		
ABZ (3 μg/ml)	80	3	2.4	5.374	2.374	98.93		
	80	3	2.4	5.390	2.390	99.58	99.71	0.860
	80	3	2.4	5.415	2.415	100.63		
	100	3	3	6.025	3.025	100.84		
	100	3	3	6.00	3.000	100.02	100.86	0.832
	100	3	3	6.051	3.051	101.70		
	120	3	3.6	6.590	3.590	99.72		
	120	3	3.6	6.635	3.635	100.98	99.72	1.133
	120	3	3.6	6.671	3.6	101.97		

[a] Preanalyzed sample found to be 3 μg/ml.
[b] Pure drug added.
[c] Total concentration found i.e. $c = a + b$.
[d] Amount found i.e. $d = c - a$.
[e] % Recovery of ABZ or PRQ = ABZ or PRQ recovery (μg/ml)/ABZ or PRQ input (μg/ml) × 100 OR $d/b \times 100$.

Table 4b
Accuracy.

Analyte	% Added	Constant amount added[a] (μg/ml)	Amount added[b] (μg/ml)	Total amount found[c] (μg/ml)	Amount found[d] (μg/ml)	% Recovery[e]	Average % recovery	% RSD
PRZ (3 μg/ml)	50	3	1.5	4.486	1.486	99.06		
	50	3	1.5	4.498	1.498	99.86	99.62	0.484
	50	3	1.5	4.499	1.499	99.93		
	100	3	3	5.950	2.950	98.33		
	100	3	3	5.990	2.990	99.66	99.55	0.011
	100	3	3	6.020	3.020	100.66		
	150	3	4.5	7.480	4.480	99.55		
	150	3	4.5	7.459	4.459	99.08	99.55	0.007
	150	3	4.5	7.528	4.528	100.62		
ABZ (3 μg/ml)	50	3	1.5	4.516	1.516	101.06		
	50	3	1.5	4.501	1.501	100.06	100.33	0.617
	50	3	1.5	4.499	1.499	99.93		
	100	3	3	6.012	3.012	100.4		
	100	3	3	5.998	2.998	99.93	99.6	0.010
	100	3	3	5.954	2.954	98.46		
	150	3	4.5	7.501	4.501	100.02		
	150	3	4.5	7.521	4.521	100.46	100.02	0.003
	150	3	4.5	7.533	4.533	100.73		

[a] Preanalyzed sample found to be 3 μg/ml.
[b] Pure drug added.
[c] Total concentration found i.e. $c = a + b$.
[d] Amount found i.e. $d = c - a$.
[e] % Recovery of ABZ or PRQ = ABZ or PRQ recovery (μg/ml)/ABZ or PRQ input (μg/ml) × 100 OR $d/b \times 100$.

Table 5
Specificity.

Analyte	% Added	Excipient amount added (mg)	Conc. found[a] (μg/ml)	% Recovery[b]	Avg. % recovery	S.D	% RSD
PRQ (10 mg)	50	5	3.008	100.28			
	50	5	3.041	101.39	100.89	0.563	0.558
	50	5	3.030	101.00			
	100	10	2.966	98.87			
	100	10	3.058	101.94	100.39	1.534	1.528
	100	10	3.010	100.36			
	150	15	2.995	99.84			
	150	15	3.032	101.08	100.74	0.791	0.78
	150	15	3.035	101.31			
ABZ (10 mg)	50	5	3.039	101.24			
	50	5	3.029	100.99	100.92	0.363	0.360
	50	5	3.015	100.53			
	100	10	3.043	101.46			
	100	10	2.999	99.97	100.81	0.759	0.753
	100	10	3.030	101.00			
	150	15	3.050	101.71			
	150	15	3.051	100.33	100.90	0.716	0.71
	150	15	3.020	100.67			

[a] The final dilution was made to 3 μg/ml and analyzed for % recovery.
[b] % Recovery of ABZ or PRQ = ABZ or PRQ recovery (μg/ml)/ABZ or PRQ input (μg/ml) × 100.

Table 6
Determination of robustness for ABZ.

Sample I.D.	Analytical condition	ABZ input (mg)	ABZ Rec. (mg)	ABZ Rec. (%)[a]	Mean Rec. ABZ (%)	S.D.	% RSD
Set-1	Flow rate: 1.02 ml/min Mobile phase pH: 3.2 Mobile phase ratio: 60:40 Column: Enable	10	9.92	99.2			
Set-2	Flow rate: 0.98 ml/min Mobile phase pH: 3.2 Mobile phase ratio: 60:40 Column: Enable	10	10.05	100.5			
Set-3	Flow rate: 1 ml/min Mobile phase pH: 3.3 Mobile phase ratio: 60:40 Column: Enable	10	9.99	99.9			
Set-4	Flow rate: 1 ml/min Mobile phase pH: 3.1 Mobile phase ratio: 60:40 Column: Enable	10	10.08	100.8	99.95	1.09	1.093
Set-5	Flow rate: 1 ml/min Mobile phase pH: 3.2 Mobile phase ratio: 58:42 Column: Enable	10	9.82	98.2			
Set-6	Flow rate: 1 ml/min Mobile phase pH: 3.2 Mobile phase ratio: 62:38 Column: Enable	10	10.11	101.1			

[a] % Recovery of ABZ = ABZ recovery (mg)/ABZ input (mg) × 100.

Table 7
Determination of robustness for PRQ.

Sample I.D.	Analytical condition	PRQ input (mg)	PRQ Rec. (mg)	PRQ Rec. (%)[a]	Mean Rec. PRQ (%)	S.D.	% RSD
Set-1	Flow rate: 1.02 ml/min Mobile phase pH: 3.2 Mobile phase ratio: 60:40 Column: Enable	10	9.85	98.5			
Set-2	Flow rate: 0.98 ml/min Mobile phase pH: 3.2 Mobile phase ratio: 60:40 Column: Enable	10	10.15	101.5			
Set-3	Flow rate: 1 ml/min Mobile phase pH: 3.3 Mobile phase ratio: 60:40 Column: Enable	10	10.02	100.2			
					100.28	1.123	1.12
Set-4	Flow rate: 1 ml/min Mobile phase pH: 3.1 Mobile phase ratio: 60:40 Column: Enable	10	10.01	101			
Set-5	Flow rate: 1 ml/min Mobile phase pH: 3.2 Mobile phase ratio: 58:42 Column: Enable	10	9.95	99.5			
Set-6	Flow rate: 1 ml/min Mobile phase pH: 3.2 Mobile phase ratio: 62:38 Column: Enable	10	10.10	101.0			

[a] % Recovery of PRQ = PRQ recovery (mg)/PRQ input (mg) × 100.

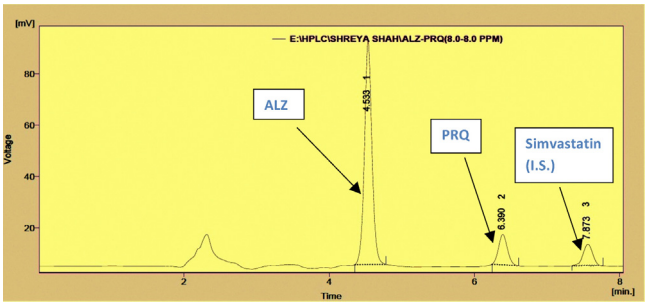

Fig. 7. Chromatogram of albendazole (4.533), praziquantel (6.39) and simvastatin as Intrnal Standard (I.S.) (7.873) (8.0 μg/ml).

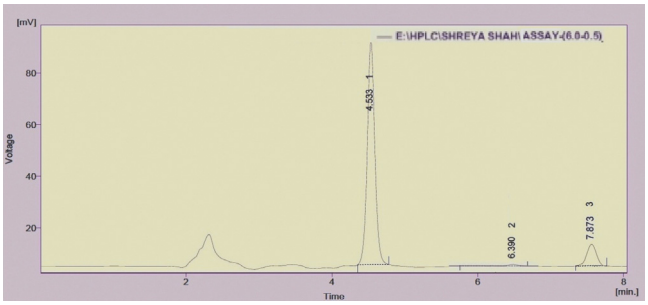

Fig. 8. Chromatogram of Albendazole and Praziquantel assay containing 6 μgm/ml of ABZ & 0.5 μg/ml of PRQ with simvastatin as an Internal Standard (IS) (7.873).

Table 8
Determination of % assay for ABZ & PRQ.

Synthetic mixture	Drug	Label claim mg/tablet	Conc. estimated (mg)	Mean conc. estimated (mg)	% Assay (w/w)±[a]	% RSD[b]
ABZ + PRQ	PRQ	25 mg	25.287 25.191 24.762 24.999 25.191 24.833	25.044	99.77 ± 0.014	0.24
	ABZ	300 mg	298.36 298.89 298.96 299.44 300.2 300.06	299.32	100.17 ± 0.0042	0.85

[a] % Recovery of ABZ or PRQ = ABZ or PRQ recovery (mg)/ABZ or PRQ input (mg) × 100 ± SD.
[b] % RSD = SD/mean × 100.

Table 9
System suitability parameters for ABZ & PRQ.

S. no.	Parameters	ABZ (min)	PRQ (min)
1	Retention time, Rt (min)	4.5 min	6.39 min
2	Capacity factor (k)	4.468	6.6875
3	Separation factor (α)	1.496	1.496
4	Theoretical plates (USP)	3388.762	9682.56
5	HETP (mm)	0.0738	0.0258
6	Resolution (Rs)	3.9426	7.0950

praziquantel. The results are summarized in Table 8, and the chromatogram is shown in Fig. 8.

3.2.8. System suitability

The system suitability test is an integral part of chromatographic analysis. It is used to verify that the resolution and reproducibility of the system are adequate for the analysis. A system suitability test according to the United States Pharmacopeial Convention was performed on chromatograms obtained for standard and test solutions to check differences in the above-mentioned parameters. The results obtained with six replicate injections of the standard solution are summarized in Table 9.

4. Conclusions

An HPLC method for albendazole and praziquantel has been developed, which is simple, precise and selective for simultaneous determination of the two drugs in bulk and in synthetic mixtures. It can be used for process control of bulk drug and formulated products in ordinary laboratories and in the pharmaceutical industry.

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